FUNGI ATTACKING SEEDS IN DRY SEED-BEDS

D. M. Griffin
School of Agriculture, University of Sydney, N.S.W., Australia

[Read 27th April, 1966]

Synopsis

Soil samples were brought to approximate equilibrium with atmospheres of 100, 95, 90, 85 and 80% R.H. The pattern of fungal colonization of wheat seeds buried in the soil samples was then compared with that occurring in seeds stored at similar humidities, and was found to be similar. The breakdown of buried seeds was due to such seed-borne fungi as members of the *Aspergillus glaucus* group and the *Penicillium chrysogenum* and the *P. citrinum* series and to various soil-borne aspergilli and penicillia, of which *P. expansum* was the most important. Only in the 80% R.H. series did most of the buried seeds survive for more than three weeks and the period was less than one week for those in the 100% and 95% R.H. series. Shaking the seeds with " New Improved Ceresan " fungicidal dust prior to planting did not enhance longevity.

Introduction

The subject of deterioration of stored grain due to fungal activity has been reviewed by Christensen (1957) who concluded that fungal invasion was the main cause of damage to the embryos of cereals. Species of *Aspergillus* and *Penicillium* are able to cause seed decay on an economically-important scale in atmospheres with a relative humidity as low as 70% (Snow, Crichton and Wright, 1944 ; Snow, 1945). Although fungi are active in soil at similar low relative humidities (Dommergues, 1962 ; Griffin, 1963*b* ; Kouyeas, 1964), there has been no study of germination reduction in seeds sown in soil with a moisture content below that of the permanent wilting point. In Australia and similar regions, however, this is a matter of some importance, for germination of seed is often retarded by low soil moisture at, or immediately after, sowing.

Materials and Methods

To control relative humidity in the experiment using soil, thin layers of air-dry sieved soil were placed in small containers in each of six vacuum desiccators, the basal portions of which contained one of the six sodium chloride solutions listed in Table 1. The desiccators were then evacuated for one week to allow the soils to attain the required relative humidity. The water contents of soil samples (Table 1) indicate that the 100% R.H. sample was at approximately permanent wilting point and that those from other humidities were considerably drier.

Sixty seeds were placed in perforated plastic containers hanging above *c.* 170 ml. solutions of sodium chloride in closed jars of 500 ml. capacity. In the experiments using soil, about 100 g. of soil, after equilibration, were also placed in the containers. After 10 weeks, a test using Clark's and Severinghaus' electrodes showed an oxygen concentration in the jars not below 19% and a carbon dioxide concentration not above 2%. Any change in the atmosphere of the jars was therefore negligible. In a small pilot experiment, variation between replicates in both the pattern of colonization and the rate of seed degeneration was also found to be negligible and the main experiments were therefore not replicated.

Table I

Moisture data for soil samples

	Relative humidity					Saturated soil	Field capacity	Permanent wilting point
	100	95	90	85	80			
Controlling salt solution (g. NaCl/100 ml. H_2O)	0	8·6	16·5	23·6	30·0	—	—	—
Suction (pF)*	—†	4·86	5·17	5·36	5·50	0	2·0	4·2
Moisture content (g. H_2O/100 g. dry soil)	21	10	9	8	7·3	63	39	18
Moisture content (% saturated) ..	33	16	14	13	11·6	100	62	29

* Relationship between soil water suction and R.H. has been given by myself (Griffin, 1963*a*, *b*) with an error in the formula. M=molecular weight of water, not density.

† Not in true equilibrium.

The soil was a black earth (Stephens, 1962) of very heavy texture (pH 8·6) from Narrabri in the north-west wheat areas of New South Wales.

The wheat seeds were of the variety Mengavi and were harvested approximately one year before the start of the experiments.

At each sampling, five seeds were taken from each relative humidity and were sterilized by successive immersions in (1) 95% alcohol (momentarily), (2) 1% silver nitrate (0·5 min.) and (3) saturated sodium chloride (3 min.). Each seed was then cut aseptically into four portions, three seeds being placed on Petri dishes containing Czapek-Dox agar (3% sucrose) and two on dishes containing Czapek-Dox Sucrose agar (20% sucrose). Seeds from the same containers were also tested for ability to germinate when placed on wet filter-paper in a Petri dish.

All experiments were conducted at 25°C (±0·5°) in a dark room and no water was deposited on the surfaces of the containers.

Table 2

Number of seeds germinating, out of ten, after various periods in controlled humidity jars

Week	Relative Humidity				
	100	95	90	85	80
1	10	10	10	10	10
2	3	6	8	9	10
3	2	2	5	7	10
4	1	3	2	7	8
5	2	0	4	3	10
7	0	2	1	2	9
9	0	0	3	2	6
13	—	0	1	0	1
17	—	—	—	—	2

Experimental

Internal microflora of seeds

The experiment was made to study the pattern of colonization of the interior of the seed by the seed-borne flora. Wheat seeds alone were therefore placed in the experimental containers.

Data on seed germination are given in Table 2. The fungi isolated from surface-sterilized seed were, with very few exceptions, species of *Aspergillus* and *Penicillium*. Owing to the presence of many intermediary forms, these fungi have not been identified at the species level but have been referred in Table 3 to the appropriate group or series (Thom and Raper, 1945 ; Raper and Thom, 1949). Within the important *A. glaucus* complex, *A. amstelodami* (Mang.) Thom and Raper, *A. chevalieri* (Mang.) Thom and Raper, *A. repens* (Cda.) De Bary and *A. ruber* (Spiek. and Brem.) Thom and Church were isolated. Other fungi occasionally isolated but not included in Table 3 were *Alternaria* sp., *Aspergillus ochraceus* Wilhelm, *A. ustus* (Bain.) Thom and Church, *Mucor* sp., *Penicillium lilacinum* Thom and *P. purpurogenum* Stoll.

TABLE 3

Aspergilli and Penicillia isolated from surface-sterilized seeds

Week	Relative Humidity				
	100	95	90	85	80
1	*P. chrysogenum*	*P. chrysogenum*			*P. chrysogenum*
2	*A. candidus* *P. chrysogenum*	*A. glaucus* *P. chrysogenum*			*P. chrysogenum*
3	*A. glaucus* *P. citrinum*	*P. chrysogenum*	*A. glaucus*		
4	*P. chrysogenum*	*A. candidus* *A. glaucus* *P. chrysogenum* *P. citrinum*	*A. glaucus* *P. chrysogenum*	*A. glaucus*	
5	*A. candidus* *A. glaucus* *A. versicolor* *P. citrinum*	*A. versicolor* *P. citrinum*	*A. candidus* *A. glaucus* *P. citrinum*		
7	*A. versicolor*	*A. glaucus* *A. versicolor* *P. chrysogenum*	*A. glaucus*	*A. flavus-oryzae* *A. glaucus*	*A. glaucus* *P. citrinum*
9	*A. flavus-oryzae* *P. chrysogenum* *P. citrinum*	*A. candidus* *A. flavus-oryzae* *A. versicolor* *P. citrinum*	*A. flavus-oryzae* *A. glaucus* *P. citrinum*	*A. glaucus* *P. citrinum*	*A. glaucus* *P. citrinum*
13	—	*A. glaucus* *A. versicolor*	*A. glaucus* *A. versicolor* *P. chrysogenum* *P. citrinum*	*A. glaucus* *P. chrysogenum* *P. citrinum*	*A. glaucus* *P. citrinum*
17	—	—	—	—	*A. glaucus* *P. chrysogenum*

Internal microflora of seeds in contact with soil

Wheat seeds were buried in small quantities of soil in the experimental containers and the pattern of colonization of the interior of the seeds was again studied.

Data on germination are given in Table 4 and on the commonest fungi isolated (identified to group or series only) in Table 5. Other fungi sometimes isolated were *Aspergillus avenaceus* G. Smith, *A. flavepes* (Bain. and Sart.) Thom and Church, *A. ochraceus* Wilh., *Cephalosporium* sp., *Fusarium* spp., *Penicillium brevi-compactum* Dierckx, *P. canescens* Sopp, *P. frequentans* Westling, *P. funiculosum* Thom, *P. oxalicum* Currie and Thom, *P. purpurogenum* Stoll, and *Rhizopus stolonifer* Fr.

An attempt to improve longevity by coating seeds with a fungicidal dust before burying was unsuccessful. The seeds were shaken-up with an excess of "New Improved Ceresan" dust (ethyl mercuric phosphate—1·3% Hg) and then buried, but the decrease in viability was very similar to that in the previous experiments and the fungicide had clearly been ineffective. The only fungus to be isolated from any of the seeds was *Penicillium expansum* Link and this species was shown in a subsidiary experiment to be unusually tolerant of the fungicide when it was added to agar media. On an agar medium containing *c.* 1 p.p.m. of the fungicide, only *P. expansum* and *P. citrinum*, of the fungi tested, suffered no check to growth whereas the growth rates of *P. chrysogenum*, *P. urticae*, *Aspergillus versicolor* and *A. ruber* were, at most, half of the normal. *P. expansum* was the only fungus little affected by a concentration of *c.* 100 p.p.m.

Table 4

Number of seeds germinating, out of ten, after various periods in soil in controlled humidity jars

Week	Relative Humidity				
	100	95	90	85	80
1	1	4	10	10	10
2	2	1	4	10	10
3	0	0	2	7	8
4	0	3	0	2	10
5	2	0	0	0	10
7	—	1	0	0	7
9	—	0	2	3	4
13	—	0	0	3	0

Discussion

The pattern of fungal colonization of the unburied seeds is similar to that described by many previous workers studying the deterioration of stored seed, with members of the *Aspergillus glaucus* group and the *Penicillium citrinum* and *P. chrysogenum* series being especially prominent. The rate of colonization increased with increasing humidity and there was little fungal invasion at 80% R.H. until five weeks had elapsed.

With the seed buried in soil, a greater variety of fungi was recorded, but the rate of deterioration was approximately the same as in stored seed. From a comparison of the fungi listed in Tables 3 and 5 it can be seen that seed destruction in soil was due to both seed-borne and soil-borne fungi, and of the latter, *Penicillium expansum* was particularly important.

In both experiments, early records of a fungus at the lower humidities presumably indicate its presence as spores or a few hyphae in the more superficial layers of the seed but still in a position shielded from the effects of surface sterilization (Hyde, 1950, 1951), so that the presence of the fungus could not be detected visually until after incubation on agar. Dead seeds, however, usually bore an external mass of conidiophores and were also heavily colonized internally. It seems reasonable to suppose that this fungal invasion was the cause of death, for cereal seeds seem to offer no resistance to fungal attack when held at moisture levels sufficient for fungal growth but insufficient for seed germination. Such a contention is also supported by the fact that the rise in respiration rates of ungerminated seeds with increase in moisture content is almost entirely due to increased respiration of fungi within, or on the surface of, the seed (Crocker and Barton, 1957).

The failure of the fungicidal dust is in accord with similar experiments with stored grain (Christensen, 1957) and should probably be attributed to the inactivity of the fungicide in the absence of free water. The isolation of *P. expansum* alone from dusted seeds cannot be interpreted as indicating that this species was the only one active in the seeds. Christensen has shown that although ' Ceresan-M ' is not toxic to fungi inside seeds, the residual fungicide on the seed coat, diffusing into the agar, is able to suppress fungal growth from the seed on to agar. In the present instance, it is therefore likely that a mixed microflora caused the seed decay but that only *P. expansum* was able to grow out on to agar.

TABLE 5

Aspergilli and Penicillia isolated from surface-sterilized seeds which had been buried in soil

Week	Relative Humidity				
	100	95	90	85	80
1	*P. chrysogenum* *P. citrinum* *P. urticae*	*P. chrysogenum* *P. citrinum*	*A. glaucus* *P. chrysogenum*	*P. citrinum*	*P. chrysogenum*
2	*P. chrysogenum* *P. urticae*	*P. chrysogenum* *P. urticae*	*P. chrysogenum* *P. urticae*	*P. chrysogenum*	*P. chrysogenum*
3	*P. expansum* *P. javanicum* *P. urticae*	*P. chrysogenum* *P. expansum*	*A. glaucus* *A. versicolor* *P. chrysogenum* *P. citrinum* *P. expansum* *P. nigricans*	*A. glaucus* *P. chrysogenum* *P. citrinum*	*A. glaucus* *P. chrysogenum* *P. citrinum* *P. rugulosum*
4	*P. expansum* *P. janthinellum* *P. rugulosum*	*P. brevi-compactum* *P. chrysogenum* *P. citrinum* *P. nigricans*	*A. glaucus* *P. chrysogenum* *P. citrinum* *P. expansum* *P. nigricans*	*A. glaucus* *P. chrysogenum* *P. expansm* *P. rugulosum* *P. urticae*	*A. flavus-oryzae* *P. chrysogenum* *P. expansum* *P. janthinellum* *P. rugulosum* *P. urticae*
5	—	*A. glaucus* *P. chrysogenum* *P. expansum* *P. javanicum*	*A. glaucus* *A. versicolor* *P. chrysogenum* *P. citrinum* *P. expansum* *P. javanicum* *P. nigricans*	*A. glaucus* *P. brevi-compactum* *P. chrysogenum* *P. expansum* *P. nigricans*	*A. versicolor* *P. chrysogenum* *P. citrinum* *P. expansum* *P. janthinellum* *P. rugulosum*
7	—	*P. chrysogenum* *P. expansum* *P. rugulosum* *P. urticae*	*P. brevi-compactum* *P. chrysogenum* *P. citrinum* *P. janthinellum*	*A. glaucus* *P. brevi-compactum* *P. expansum*	
9	—	*A. versicolor* *P. brevi-compactum* *P. chrysogenum* *P. expansum*	*A. versicolor* *P. chrysogenum* *P. expansum*	*A. glaucus* *A. versicolor* *P. expansum* *P. javanicum*	*A. versicolor*
13	—	*P. chrysogenum* *P. expansum* *P. rugulosum* *P. urticae*	*A. glaucus* *P. expansum*	*A. glaucus* *P. expansum* *P. javanicum*	*A. glaucus* *P. brevi-compactum* *P. chrysogenum* *P. janthinellum*

Seed decay was rapid in soils at about permanent wilting point or somewhat drier and only in the driest soils did seeds survive for more than three weeks. It seems unlikely that any seed treatment will improve longevity under these conditions, for much of the decay is due to fungi carried inside the testa and at least some of the soil-borne fungi are likely to be tolerant of fungicidal dusts, especially under dry conditions where the compounds are relatively inactive.

Acknowledgements

I wish to thank Miss E. M. Palmer and Miss C. M. Siddle for laboratory assistance. This work was done whilst I was in receipt of grants from the Wheat Industry Research Council and the N.S.W. Wheat Industry Research Committee.

References

Christensen, C. M., 1957.—Deterioration of stored grains by fungi. *Bot. Rev.*, 23 : 108–134.

Crocker, W., and Barton, L. V., 1957.—" Physiology of seeds ". Chronica Botanica, Waltham.

Dommergues, Y., 1962.—Contribution a l'étude de la dynamique microbienne des sols en zone semi-aride et en zone tropicale sèche. *Ann. Agron. Paris*, 13 : 265–324, 379–469.

Griffin, D. M., 1963*a*.—Soil moisture and the ecology of soil fungi. *Biol. Rev.*, 38 : 141–166.

————, 1963*b*.—Soil physical factors and the ecology of fungi. III. Activity of fungi in relatively dry soil. *Trans. Brit. mycol. Soc.*, 46 : 373–377.

Hyde, M. B., 1950.—The subepidermal fungi of cereal grains. I. A survey of the world distribution of fungal mycelium in wheat. *Ann. appl. Biol.*, 37 : 179–186.

Hyde, M. B., and Galleymore, H. B., 1951.—The subepidermal fungi of cereal grains. II. The nature, identity and origin of the mycelium in wheat. *Ann. appl. Biol.*, 38 : 348–356.

Kouyeas, V., 1964.—An approach to the study of moisture relations of soil fungi. *Plant and Soil*, 20 : 351–363.

Raper, K. B., and Thom, C., 1949.—" A manual of the Penicillia ". Williams and Wilkins, Baltimore.

Snow, D., 1945.—Mould deterioration of feeding stuffs in relation to humidity of storage. III. The isolation of a mould species from feeding stuffs stored at different humidities. *Ann. appl. Biol.*, 32 : 40–44.

Snow, D., Crichton, M. H. G., and Wright, N. C., 1944.—Mould deterioration of feeding stuffs in relation to humidity of storage. I. The growth of moulds at low humidities. *Ann. appl. Biol.*, 31 : 102–110.

Stephens, C. G., 1962.—" A manual of Australian soils ". C.S.I.R.O., Melbourne.

Thom, C., and Raper, K. B., 1945.—" A manual of the Aspergilli ". Williams and Wilkins, Baltimore.